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DPX-MP062—a Potent Compound for Controlling the Egyptian Cotton Leafworm *Spodoptera littoralis* (Boisd.)†

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Abstract: The effects of DPX-MP062 [methyl 7-chloro-2,3,4*a*,5-tetrahydro-2-[methoxycarbonyl(4-trifluoromethoxyphenyl)carbamoyl] indeno[1,2-*e*][1,3,4] oxadiazine-4*a*-carboxylate] a broad-spectrum insecticide with a novel mode of action, on the Egyptian cotton leafworm, *Spodoptera littoralis*, were studied in laboratory experiments. Egg hatch was affected by high concentrations (125 mg AI litre^{-1}) of DPX-MP062. Larvae that hatched from treated eggs were significantly affected at concentrations of 12·5 mg AI litre^{-1} and greater. Larvae were fed castor bean leaves treated with DPX-MP062; 1st-instar larvae were the most susceptible development stage. Pupation and adult formation were determined in assays with 5th-instar larvae. There was strong suppression of adult formation; 65 and 91% at 0·5 and 0·75 mg AI litre^{-1}, respectively. Highly affected larvae died before pupation; slightly affected ones reached pupation 2–4 days later, were smaller than larvae in the untreated control, and were sometimes unable to develop into normal adults. Comparatively high concentrations (50 and 100 mg AI litre^{-1}) of the test compound were necessary to affect adults by ingestion, but no effects from contact application could be determined at a concentration of 100 mg AI litre^{-1}. © 1998 Society of Chemical Industry

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1 INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) is polyphagous, and, in cotton, feeds on all parts of the plant. Although the levels of field infestations have decreased in recent years, *S. littoralis* is still an important pest in cotton-growing areas of Africa, Asia and Europe.[1,2]

At present, *S. littoralis* is effectively controlled with benzoylphenyl ureas (chitin synthesis inhibitors), but it has developed resistance to organophosphate and pyrethroid insecticides.[3,4] Furthermore, resistance to benzoylphenyl ureas in other lepidopteran species is not unknown. For example, the codling moth, *Cydia pomonella* (L.), has developed 370-fold resistance to the benzoylphenyl urea diflubenzuron, and cross-resistance to two others (teflubenzuron, 7-fold tolerance and triflumuron, 102-fold resistance).[5] New insecticides are necessary for continued successful control of *S. littoralis*

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in the future, should resistance to benzoylphenyl ureas arise.

DPX-MP062 is an experimental mixture (approximately 3 : 1) of the *S* and *R* isomers of methyl 7-chloro-2,3,4*a*,5-tetrahydro-2-[methoxycarbonyl(4-trifluoromethoxyphenyl)carbamoyl]indeno[1,2-*e*][1,3,4]oxadiazine-4*a*-carboxylate. The *S*-isomer (for which the common name indoxacarb is proposed) is the more insecticidally active of the two. The isomers represent a new class of compound and are considered to have broad-spectrum insecticidal activity and yet be environmentally soft. They have a novel mode of action and thus lack cross-resistance to a wide range of standard insect control compounds. The active isomer of the compound blocks sodium channels in nerve cells leading to poor coordination, paralysis and death of the insect.[6–8] The bioactivation of the parent isomer to the decarbomethoxylated metabolite, which blocks the sodium channel, has been described in three *Spodoptera* species.[9] The compound has been shown to have minimal effect on beneficial mites and insects.[6,7] We have evaluated the potential of DPX-MP062 for the control of *S. littoralis*, an important pest of cotton and other field crops.

2 EXPERIMENTAL METHODS

2.1 Chemical

An experimental DPX-MP062 150 g litre^{-1} suspension concentrate (SC; Du Pont de Nemours France SA) was diluted with deionized water to the desired concentrations.

2.2 Insect rearing

Egyptian cotton leafworm larvae were reared on freshly collected castor bean leaves (*Ricinus communis* L.) under standard laboratory conditions of 24 ($\pm$1)°C, 65% RH and a 14 : 10 h light : dark photoperiod.[5] Adults were maintained in 3–4 litre jars on 10% saccharose solution. Paper towelling was provided for oviposition.

2.3 Immature bioassays

Bioassays were carried out on eggs (0–24 h old), 1st- (0–24 h old), 3rd- (0–24 h after ecdysis, weighing 11 ($\pm$1) mg) and 5th-instar larvae (0–24 h after ecdysis, weighing 175 ($\pm$15) mg), and with adults (0–24 h old). Initial bioassays, with a broad range of DPX-MP062 concentrations, were performed on all stages to determine appropriate endpoint concentrations.

Eggs, laid on paper towels, were dipped in aqueous dilutions of the test formulation, or in deionized water as control. After drying for 2 h at room temperature, the treated eggs were stored in plastic Petri dishes. Three days later, untreated castor bean leaves were added to the Petri dishes for larvae to feed on. Egg-hatch and larval survival were monitored daily; results were recorded six days after application. Results are based on eight to 20 replicates of 26 to 87 *S. littoralis* eggs each.

In other assays, larvae were exposed to castor bean leaves treated with aqueous dilutions of the test formulation, or with deionized water as control. Leaves were dipped for 10 s in the appropriate solution, dried for 2 h, then offered to 1st- and 3rd-instars for four days, and to 5th-instars for three days. After these time intervals the treated leaves were replaced by untreated ones. Ten 1st-instar larvae were kept in plastic Petri dishes, and 3rd- and 5th-instars were held in ventilated plastic boxes with 10 and five larvae each, respectively. The boxes contained sawdust to reduce moisture. Larval mortality was determined after completion of the subsequent ecdysis (1st- and 5th-instars at day 6, 3rd-instars at days 4 and 6 after exposure to treated material). Weight gains of the 3rd- and 5th-instar larvae were determined six and three days, respectively, after feeding on treated leaves. Pupation and adult formation of 5th-instars were evaluated 12 and 30 days, respectively, after the start of the assay. Results are based on 16 to 24 replicates of 10 1st-instars, six to 12 replicates of 10 3rd-instars, and 16 replicates of five 5th-instar *S. littoralis* larvae each.

2.4 Adult bioassays

Effects of ingestion of DPX-MP062 by adults were studied by allowing adults, from eclosion, to feed on 10% saccharose solution containing 50 or 100 mg AI litre^{-1} of the test compound, or 10% saccharose solution as control. Male and female moths were kept in separate jars containing five individuals each. Mortality was checked daily for five days. Solutions were changed every second day to prevent fungal growth. Results are based on six to 14 replicates.

To study the contact effect of DPX-MP062, the jars housing adults were lined with treated or untreated paper towels. Paper towels were dipped for 10 s in a solution of 100 mg AI litre^{-1} of the test compound, or water as control, then dried for 2 h. Five male or female, recently eclosed, moths were kept in each jar and fed on 100 g litre^{-1} saccharose solution. Mortality was checked and solutions were changed as described above. Assays were replicated seven times.

2.5 Residual toxicity

Cotton leaves, collected in August 1997 from field plots located at Bet Dagan (central Israel), treated with 5 or 25 mg AI litre^{-1} of DPX-MP062, were exposed to 3rd-

instar *S. littoralis* larvae for four days, to determine residual toxicity. Leaves were collected on days 1, 3, 7, 14 and 21 after treatment. Untreated leaves were collected from an unsprayed plot and used as control.

2.6 Data analysis

Data were corrected for control mortality,[10] and analysis of variance was used for evaluating probability of significant differences between samples. Means were separated according to the Student–Newman–Keuls (SNK) multiple range test ($P = 0{\cdot}05$). Data presented as percentages were transformed using angular transformation before statistical analysis. The slope and LC values of the test compound for different larval stages were determined by probit regression using the POLO analysis procedure.[11]

3 RESULTS AND DISCUSSION

3.1 Effect on eggs and young larvae

The effect on egg-hatch and larval survival was determined by dipping newly laid eggs in various concentrations of DPX-MP062, and in water as control. High concentrations of the compound were required in order to affect egg-hatch of *S. littoralis* (Table 1). Inhibition of egg-hatch of 47% was obtained with 125 mg AI litre^{-1}. The relatively high concentrations required for suppression of egg-hatch are probably due to the compound not being able to penetrate the eggshell. Some benzoylphenyl ureas, such as diflubenzuron and trifluron, are toxic to *S. littoralis* eggs.[12–15]

Larvae from treated eggs were significantly affected at concentrations of 12·5 mg AI litre^{-1}. Effective control of these larvae was only obtained at concentrations of 75 and 125 mg AI litre^{-1} (2 and 1% survival, respectively, after six days). The relatively high mortality of the larvae probably resulted from their eating the egg shell and contacting the compound at a very young stage. It was observed that the larvae died very soon after eclosion.

TABLE 1
Effect of DPX-MP062 on Egg-Hatch and Larval Survival of *Spodoptera littoralis*

DPX-MP062 concentration (*mg litre*$^{-1}$)	*Egg-hatch*,[a] (%) (±*SEM*)	*Larval survival*,[a] (%) (±*SEM*)
0	59 (±4)a	59 (±4)a
12·5	53 (±8)ab	34 (±8)b
25	42 (±4)ab	10 (±4)c
75	50 (±5)ab	2 (±1)d
125	28 (±6)b	1 (±0)d

[a] Results are averages of eight to 20 replicates of 26 to 87 *S. littoralis* eggs (0 to 24-h-old) each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test). Angular transformation was used before statistical analysis.

3.2 Larval mortality

First-, 3rd- and 5th-instar *S. littoralis* larvae were fed on castor bean leaves treated with various concentrations of the test compound. All larval instars were at least 20-fold more susceptible to the compound than were eggs (Table 1, 2, 3). According to LC_{50} and LC_{90} values, 1st-instars are the most susceptible (Table 3). Increases in mortality of 3rd-instar larvae were seen even after replacing treated leaves with untreated ones; heavily affected larvae were not able to recover and died at a later stage (Table 2, six-day mortality).

3.3 Ingestion effects on weight gain

Larvae were weighed before exposure to treated leaves and again six days (3rd-instars) or three days (5th-instars) after exposure. Control 3rd- and 5th-instar larvae, fed untreated leaves, were weighed after similar time intervals. Larvae that fed on DPX-MP062-treated leaves weighed less than control larvae (Table 4); the higher the concentration of DPX-MP062, the lower the larval weight. The weight gain of 3rd-instars exposed to 0·1 mg AI litre^{-1} DPX-MP062 was 55% of the untreated larval weight, while larvae treated with 0·2 mg AI litre^{-1} reached only 15% of the untreated larval weight. Fifth-instar larvae exposed to 0·25 mg AI litre^{-1} reached 45% of the larval weight of the untreated control, and many of those exposed to 0·5 mg AI litre^{-1} experienced weight loss. Larvae of both stages were unable to maintain normal growth when fed

TABLE 2
Effect of DPX-MP062 on Mortality of 3rd-Instar *Spodoptera littoralis* Larvae

DPX-MP062 concentration (*mg litre*$^{-1}$)	*Mortality*[a] (%) (±*SEM*) *4 days*	*6 days*
0	0a	0a
0·1	2 (±2)a	3(±2)a
0·2	22 (±2)b	41(±3)b
0·4	28 (±3)b	56(±5)c
0·8	44 (±5)c	78(±6)d
1·6	55 (±9)c	100e

[a] Results are averages of six to 12 replicates of 10 3rd-instar *S. littoralis* larvae each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test). Angular transformation was used before statistical analysis.

TABLE 3
Slopes and LC Values of DPX-MP062 on Different Larval Stages of *Spodoptera littoralis*[a]

	1st-instar larvae	*3rd-instar larvae*	*5th-instar larvae*
Slope (±SEM)	4·02 (±0·25)	2·33 (±0·21)	5·39 (±0·78)
LC_{50} (mg litre^{-1}) (95% FL)	0·11 (0·08–0·16)	0·32 (0·18–0·52)	0·47 (0·41–0·52)
LC_{90} (mg litre^{-1}) (95% FL)	0·23 (0·16–0·77)	1·15 (0·69–6·46)	0·81 (0·72–0·98)

[a] Results, taken at day 6 after start of assay, are based on 24 replicates of 10 1st-instars, 6–12 replicates of 10 3rd-instars, and 16 replicates of five 5th-instar larvae each. Five to six concentrations were used for determining slopes and LC values. Calculations were done by POLO analysis procedure.[10]

TABLE 4
Activity of DPX-MP062 on 3rd- and 5th-instar *Spodoptera littoralis* Larvae Expressed as Weight Gain per Larva

3rd-instar larvae		*5th-instar larvae*	
DPX-MP062 concentration (mg litre^{-1})	*Weight gain[a] (mg) (±SEM)*	*DPX-MP062 concentration (mg litre^{-1})*	*Weight gain[a] (mg) (±SEM)*
0	154 (±15)a	0	312 (±11)a
0·1	86 (±14)b	0·25	108 (±14)b
0·2	23 (±3)c	0·5	25 (±6)c
0·4	11 (±2)c	0·75	3 (±4)c
0·8	1 (±0)c	1	1 (±3)c

[a] Results are averages of six to 12 replicates of 10 3rd-instars and 16 replicates of five 5th-instar *S. littoralis* larvae each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test).

on leaves treated with 0·8 mg AI litre^{-1}, and almost half of the individuals lost weight.

This weight loss as a result of feeding on DPX-MP062 is in contrast to the action of benzoylphenyl ureas which, although effective when ingested, exhibit significant contact activity. The effect of benzoylphenyl ureas occurs only at the subsequent moult;[16,17] that is, larvae continue to feed and gain weight during the inter-mould period. Fisk and Wright[18] reported no significant weight loss 24 h after 3rd-instar *S. littoralis* were exposed to teflubenzuron.

3.4 Effect on pupation and adult formation

Pupation and adult formation of 5th-instar larvae were determined 12 and 30 days, respectively, after the trial started. There was no significant difference between control larvae and those exposed to 0·25 mg AI litre^{-1}; approximately 80% of the larvae became adults (Table 5). Suppression of adult formation of 65 and 91% occurred at concentrations of 0·5 and 0·75 mg AI litre^{-1}, respectively. Highly affected larvae were unable to increase weight and died before pupation. Slightly affected larvae reached pupation two to four days later than larvae in the untreated control, but pupae were smaller and sometimes unable to develop into normal adults.

3.5 Effect of ingestion and contact exposure on adults

The effect of DPX-MPO62 by ingestion on mortality of *S. littoralis* adults was tested by exposing male and female adults to saccharose solutions containing different concentrations of the test compound. A significant

TABLE 5
Effect of DPX-MP062 on Pupation and Adult Formation of *Spodoptera littoralis*

DPX-MP062 concentration (mg litre^{-1})	*Pupation[a] (%) (±SEM)*	*Adult formation[a] (%) (±SEM)*
0	93 (±3)a	83 (±4)a
0·25	88 (±4)a	80 (±4)a
0·5	39 (±7)b	35 (±7)b
0.75	14 (±5)c	9 (±4)c

[a] Results are averages of 16 replicates of five 5th-instar larvae each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test). Angular transformation was used before statistical analysis.

effect was observed at concentrations of 50 and 100 mg AI litre^{-1} resulting in 35 and 60% mortality after three days of feeding, and in 93 and 87% mortality after five days of feeding (Table 6). Although adults were strongly affected five days after exposure to the compound, these effects were very mild when compared to the ingestion effects on the larval stages (Table 3). The potency of DPX-MP062 was similar between the sexes of *S. littoralis* adults. No contact effect was observed on adults at concentrations up to 100 mg AI litre^{-1}.

3.6 Residual toxicity

Leaves were collected from cotton field plots sprayed with 5 or 25 mg AI litre^{-1} DPX-MP062 and fed to 3rd-instar *S. littoralis*. There was strong residual toxicity in leaves sprayed with 25 mg AI litre^{-1} (over 95% mortality) until day 7 (Table 7). Lower residual toxicity caused 50% mortality at day 21. A concentration of 5 mg AI litre^{-1} did not have sufficient residual activity to affect 3rd-instars. The residual toxicity of DPX-MP062 largely corresponded with results recently obtained in assays with an experimental benzoylphenyl urea.[19]

4 CONCLUSION

Spodoptera littoralis damages cotton plants by feeding on leaves, squares and bolls.[1] Insecticides used for control of *S. littoralis* ideally should suppress larval feeding and disrupt the life cycle of the pest, without harming natural enemies or the environment. Furthermore, there is urgent need for effective insecticides that have no cross-resistance with existing ones.

Present results verify direct lethal effects of DPX-MP062 on larval stages of *S. littoralis* (treated larvae were unable to pupate or form adults), and demonstrate indirect effects as well: particularly, weight loss during the stadium in which they fed. Residual toxicity on plant leaves was observed for up to 21 days. The lack of contact effect on adults at concentrations up to 100 mg AI litre^{-1}, and the slight effect on eggs, indicate a weak contact effect of the compound.

There is an established insecticide resistance management (IRM) programme for many of the insect pest

TABLE 6
Ingestion and Contact Effect of DPX-MP062 on Adult *Spodoptera littoralis*

DPX-MP062 concentration (mg litre^{-1})	*Adult mortality[a] (%) (±SEM)*			
	Ingestion		*Contact*	
	3 days	*5 days*	*3 days*	*5 days*
0	9 (±3)a	13 (±3)a	6 (±4)a	20 (±9)a
50	35 (±6)b	93 (±5)b		
100	60 (±14)c	87 (±4)b	6 (±4)a	17 (±7)a

[a] Results are averages of 6–14 replicates of five adults each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test). Angular transformation was used before statistical analysis.

TABLE 7
Residual Toxicity of DPX-MP062 against 3rd-instar *Spodoptera littoralis* Larvae under Field Conditions

DPX-MP062 concentration (mg litre^{-1})	*Mortality[a] (%) (±SEM)*				
	Days after treatment				
	1	*3*	*7*	*14*	*21*
0	4 (±3)a	9 (±3)a	9 (±2)a	5 (±3)a	3 (±2)a
5	56 (±8)b	35 (±6)b			
25	98 (±1)c	100c	96 (±2)b	43 (±4)b	50(±4)b

[a] Results are averages of 10 replicates of 10 3rd-instar larvae each. Within columns, means followed by the same letter do not differ significantly at $P = 0{\cdot}05$ (SNK multiple range test). Angular transformation was used before statistical analysis.

species on cotton in Israel.[1] With the development of new compounds (such as DPX-MP062) as alternatives to the benzoylphenyl ureas currently in use,[3] *S. littoralis* could be successfully included in the management programme.

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